

AMENDMENTS TO THE DRAWINGS

Please replace the drawing of Figure 2 with the attached sheet corresponding thereto.

REMARKS**Status of the Claims**

Claims 1 and 3-16 are pending in the present application. Claim 2 was previously canceled. Claims 1, 15, and 16 are amended. Support for these amendments is found throughout the application as originally filed including paragraph [0017] of the originally filed application. Claim 3 is also amended to depend on a pending claim. No new matter is entered by way of this amendment. Reconsideration is respectfully requested.

Drawings

Applicants submit herewith a Replacement Drawing, which corresponds to Figure 2. The Replacement Drawing is submitted per the Examiner's request, *see* below. No new matter is added by the Replacement Drawing.

Substance of the Interview

Applicants and Applicants' representative thank the Examiner for extending the courtesy of an interview on March 4, 2010. During the interview, the rejections under 35 U.S.C. § 102 and 35 U.S.C. § 103(a) and the provisional non-statutory obviousness type double patenting rejections were discussed. The Examiner noted that the Exhibits, which were submitted in the response filed on September 2, 2010, should be submitted in Declaration form. The Examiner further indicated that Figure 2 of the originally filed application, which compares gene transfer efficiency using the syringe method versus the centrifugation method, should be resubmitted since the copy in the file wrapper is somewhat unclear. The Examiner further stated that, if Applicants amend the claims to describe the pressurization methods as described in paragraph [0017] of the originally filed application, and provide the above-described Declaration and Replacement Figure, the application is likely to be allowed. Provided herewith is the Ishida Declaration, describing the previously submitted Exhibits, and a Replacement Figure 2. The claims are amended as discussed during the interview.

Non-Statutory Double Patenting

Claims 1, 3-9, and 12-16 are provisionally rejected on the ground of non-statutory obviousness-type double patenting as allegedly unpatentable over claims 1, 12, 14-15, 17-18, 20-21, and 23-24 of co-pending U.S. Application No. 10/089,695, *see Office Action*, pages 2-3.

Claims 1, 3-9, and 12-16 are also provisionally rejected on the ground of non-statutory obviousness-type double patenting as allegedly unpatentable over claims 22-29 of co-pending U.S. Application No. 10/089,696, *see Office Action*, page 3.

The Examiner states that the pressure described in the instant claims, *i.e.*, 1.7 to 10 atmospheres, is inherent or obvious in view of the claims of the co-pending applications. That is, the Examiner believes that the centrifugation speeds and durations described in the co-pending application claims, *e.g.*, 1000G to 150,000G from 1 second to four hours, would have necessarily resulted in plant material being pressurized at from 1.7 to 10 atmospheres.

As amended, independent claims 1, 15, and 16 do not encompass centrifugation. The amended independent claims specify that the pressurization means is selected from “i) combining syringes, holding the syringes by a clamp and tightening the clamp; ii) supplying a gas into a vessel containing a plant tissue; or iii) submerging a plant tissue bag sealed against the outside air in a liquid.” None of the claims in the cited patent applications teach or suggest these elements. Accordingly, the independent claims are not obvious over the claims in U.S. Application No. 10/089,695 or U.S. Application No 10/089,696. Dependent claims 3-9 and 12-14, which incorporate the elements of independent claim 1, also are not rendered obvious by the claims in the cited patent applications. Withdrawal of the rejections is respectfully requested.

Issues Under 35 U.S.C. §102***Hiei I or Hiei II***

Claims 1, 3-6, 8-9, and 12-16 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by European Patent Application No. 1,306,440 to Hiei *et al.*, (“Hiei I”), *see Office Action*, pages 4-5. Claims 1, 3-9, and 12-16 are also rejected under 35 U.S.C. § 102(b), as allegedly anticipated by European Patent Application No. 1,306,441 to Hiei *et al.* (“Hiei II”), *see Office Action*, page 5. The Examiner states that Hiei I teaches centrifuging embryos in liquid for 10 minutes at 100G to 200,000G, followed by selection of transformants. The Examiner further

asserts that Hiei II describe enhancing plant transformation by heating and centrifuging plant embryos in a liquid at speeds ranging from 100G to 200,000G for 1 or 30 or 60 minutes. According to the Examiner, an ordinary artisan would have recognized that submersion and centrifugal force inherently exert the claimed ranges of pressure on the plant material to which it is applied.

As noted above, independent claims 1, 15, and 16 do not encompass centrifugation as described in Hiei I and Hiei II. The amended independent claims specify that the pressurization means is selected from i) combining syringes, holding the syringes by a clamp and tightening the clamp; ii) supplying a gas into a vessel containing a plant tissue; or iii) submerging a plant tissue bag sealed against the outside air in a liquid.

In view of the foregoing, the cited references do not anticipate the independent claims. Dependent claims 3-6, 8-9, and 12-14, which incorporate the elements of independent claim 1, also are not anticipated by the cited references. Withdrawal of the rejection is respectfully requested.

Cheng

Claims 1, 3-6, 10, and 13-16 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Cheng *et al.*, *Plant Cell Reports*, 1996, 16:127-132, (“Cheng”), *see Office Action*, pages 5-6. The Examiner states that Cheng describes enhancing transformation in dicots by submerging callus in a carborundum-containing liquid and vortexing the tubes for one minute. According to the Examiner, an ordinary artisan would have recognized that submersion in a liquid, vortexing, and collision by carborundum particles would inherently exert pressure on the submerged material.

As noted above, the independent claims are amended to specify that the pressurization means is selected from i) combining syringes, holding the syringes by a clamp and tightening the clamp; ii) supplying a gas into a vessel containing a plant tissue; or iii) submerging a plant tissue bag sealed against the outside air in a liquid. Cheng does not describe these means for pressurizing plant material. Accordingly, Cheng fails to describe all of the elements of the instant claims. In view of the foregoing, the claims are not anticipated by Cheng. Withdrawal of the rejection is respectfully requested.

Issues Under 35 U.S.C. §103***Hiei I or Hiei II***

Claims 1, 3-6, 8-9, and 12-16 are rejected under 35 U.S.C. § 103(a) as allegedly obvious over Hiei I, *see Office Action*, pages 6-8. Claims 1, 3-6, 8-9, and 12-16 are also rejected under 35 U.S.C. § 103(a) as allegedly obvious over Hiei II. *see Office Action*, pages 8-9.

As noted above, neither Hiei I nor Hiei II describes that the pressurization means is selected from i) combining syringes, holding the syringes by a clamp and tightening the clamp; ii) supplying a gas into a vessel containing a plant tissue; or iii) submerging a plant tissue bag sealed against the outside air in a liquid. Instead, Hiei I discloses centrifugation of plant material. Accordingly, neither Hiei I nor Hiei II teach or suggest all of the elements of the instant claims.

Applicants further submit that the instantly claimed methods result in advantages that could not have been expected by an ordinary artisan at the time of the invention. That is, the present application teaches that pressurizing plant material according to the claimed methods results in gene transfer efficiency that is comparable or superior to centrifugation, *see especially*, Replacement Figure 2 and Example 2.

In view of the foregoing, the claims are not rendered obvious by Hiei I or Hiei II. Withdrawal of the rejection is respectfully requested.

Cheng

Claims 1, 3-6, 10-11, and 13-16 are further rejected under 35 U.S.C. § 103(a) as allegedly obvious over Cheng, *see Office Action*, pages 9-11.

As noted above, Cheng does not describe pressurizing plant material by i) combining syringes, holding the syringes by a clamp and tightening the clamp; ii) supplying a gas into a vessel containing a plant tissue; or iii) submerging a plant tissue bag sealed against the outside air in a liquid. Instead, Cheng teaches an *Agrobacterium*-mediated transformation based upon *wounding* of cultured embryogenic tissues with carborundum in a liquid phase, *see* page 127, left column, lines 4-7 of Cheng. On page 128 under the section entitled "Materials and Methods" under the subheading "Plant transformation and regeneration", the Cheng reference states that carborundum-*wounded* calli, were submerged in the culture of *A. tumefaciens* for 5 minutes, *emphasis added*. As noted in the Ishida Declaration, the pressurization methods

described in the instant claims do not result in wounding. Accordingly, Cheng does not teach or suggest all of the elements of the instant claims.

Furthermore, as evidence of superior results weighing against the allegation of obviousness, the Ishida Declaration demonstrates that pressurizing plant material by, for example, combining syringes, holding the syringes by a clamp and tightening the clamp, as described in the instant claims, results in considerably less cell death than pressurization by sonication, *see* Ishida Declaration.

In particular, the Ishida Declaration describes the percentage of cell death observed when plant cells are treated with 10 atmospheres pressure using the syringe means described in the instant claims or sonication. Cell death and cell survival were compared in the two populations. As described in the Ishida Declaration, more than 70% of cells treated with sonication for 2 seconds died. In contrast, cells treated with 10 atmosphere pressure for 15 minutes using a pressurization means specified in the instant claims demonstrated a survival rate comparable to that of cells without any treatment. Accordingly, cell death due to pressure treatment was not observed.

Based upon the foregoing, injury (damage) to plant cells resulting from pressure treatment using the claimed methods is low in comparison to cells, which were wounded by sonication. Thus, the claimed methods allow for an increase in gene transfer efficiency *via* application of appropriate pressure to a plant tissue without injuring the plant material. Applicants submit that these benefits would not have been expected by an ordinary artisan at the time of the invention.

In view of the foregoing, the instant claims are not rendered obvious by Cheng. Withdrawal of the rejection is respectfully requested.

Applicants wish to reiterate that the unobviousness of the invention is further supported by the working examples in the originally filed application, which provide experimental results of pressurization under the following conditions:

Example 1 (6)

2.4 atmospheres (+ 1.4 atmospheres), 15 minutes
4.2 atmospheres (+ 3.2 atmospheres), 15 minutes
7.6 atmospheres (+ 6.6 atmospheres), 15 minutes

Example 1 (7)

7.6 atmospheres (+ 6.6 atmospheres), 1 seconds
7.6 atmospheres (+ 6.6 atmospheres), 3 seconds
7.6 atmospheres (+ 6.6 atmospheres), 5 seconds
7.6 atmospheres (+ 6.6 atmospheres), 60 seconds

In addition, the Ishida Declaration provides supplemental experimental data, which further demonstrates support for the pressure ranges and time periods of pressurization specified in the instant claims.

Time

Shorter times-6 atmospheres, 1 seconds
Longer times-8 atmospheres, 30 minutes

Pressure

Lower pressure-2 atmospheres, 15 minutes.
Higher pressure-10 atmospheres, 15 minutes

Applicants further direct the Examiner's attention to Figure 1 of the Ishida Declaration, which depicts treatment of plants at 6 atmospheres for 1 second. This treatment resulted in GUS expression, which was more than three times the GUS expression of the control that did not receive the pressurization treatment. That is, a pressurization treatment of only 1 second was sufficient to promote the introduction of a gene into a plant material.

In view of the above, the claims are allowable. Accordingly, a notice of allowance is respectfully requested.

CONCLUSION

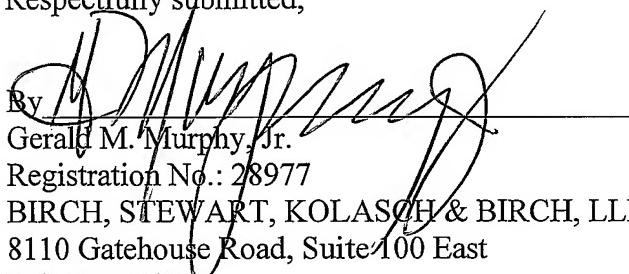
As noted above, Applicants believe the pending application is in condition for allowance.

Nevertheless, should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact L. Parker, PhD, Registration No. 46,046 at the telephone number of the undersigned below to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Director is hereby authorized in this, concurrent, and future replies to charge any fees required during the pendency of the above-identified application or credit any overpayment to Deposit Account No. 02-2448.

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Respectfully submitted,

By 
Gerald M. Murphy, Jr.
Registration No.: 28977
BIRCH, STEWART, KOLASCH & BIRCH, LLP
8110 Gatehouse Road, Suite 100 East
P.O. Box 747
Falls Church, VA 22040-0747
703-205-8000

Attachment: Ishida Declaration
Replacement Drawing